THE CHANGE IN THE HYDROGEN-ION CONCENTRATION OF VARIOUS MEDIUMS DURING HEATING IN SOFT AND PYREX GLASS TUBES

J. R. Esty and P. H. Cathcart

From the Research Laboratory, National Canners Association, Washington, D. C.

An exact knowledge of the reaction of a medium can be gained only from a determination of its hydrogen-ion concentration. It is a well established fact that sterilization of a medium or subjection of certain organic solutions to high temperatures for long periods increases their acidity. The presence in such mediums of the so-called "buffers," however, prevents rapid changes in the hydrogen-ion concentration, even if the titratable acidity is considerably altered.

In order to determine accurately the thermal death point of heat resistant spores at high temperatures it is of utmost importance to control all the factors which influence it. In a previous paper [1] it has been shown that even a slight change in the hydrogen-ion concentration greatly affects the thermal death point. It is necessary, therefore, in the determination of thermal death points to heat under conditions which will maintain a constant hydrogen-ion concentration throughout the entire period.

It is a well-known fact that heating solutions in soft glass tubes causes a certain amount of alkali to go into solution. Russell, Nichols and Stimmel [2] have found that the reaction of typhoid vaccine may vary considerably when held in soft glass containers at room temperature and depends on two factors; the amount of soluble alkali in the glass containers, and the amount of carbon dioxide absorbed from the air. They state that in itself the typhoid bacillus does not change the ordinary reaction of the medium (P_H-7.4-7.6) in which there is no sugar which is fermented by the typhoid bacillus. If the vaccine, however, is put in soft glass containers, it becomes alkaline.

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[1] W. D. Bigelow and J. R. Esty: The Thermal Death Point in Relation to Time of typical Thermophilic Organisms, Jour. Infect. Dis., 1920, 27, p. 602.

[2] Military Surgeon, 1920, 47, p. 539.

The following results reported by them are self-explanatory:

1. Vaccine placed in soft glass ampules in 1911.......... 9.26
2. Vaccine placed in soft glass ampules in 1911.......... 9.70
3. Vaccine placed in hard glass ampules in 1911.......... 8.10
4. Vaccine placed in hard glass ampules in 1919.......... 7.4
5. Vaccine placed in hard glass ampules in 1919.......... 7.6

In a personal communication with K. F. Meyer he states that there is a similar condition in bile which has been held in soft glass containers for some time.

Hard glass tubes have been suggested as a means of controlling this important factor during the heat treatment.

The object of this investigation is to determine the effect on the hydrogen-ion concentration of heating "unbuffered" solutions and solutions rich in "buffers" for long periods and at high temperatures in soft and hard glass tubes. In the article by Bigelow and Esty [1] results are given on the thermal death points of spores of certain thermophilic organisms when heated at different temperatures in food juices. These juices were heated in soft glass tubes and electrometric measurements made at definite intervals to determine the change in the hydrogen-ion concentration during the heating. At the same time determinations were made on the same juices heated for similar periods in hard glass tubes. Experiments were also conducted in which various solutions were heated in soft and hard glass tubes and the effect of the heating on the hydrogen-ion concentration noted.

A few results are also given in this paper to show the difference in the time necessary to destroy a definite suspension of spores in corn juice when heated in hard and soft glass tubes.

PREPARATION OF TUBES PRIOR TO HEATING

The soft and hard glass tubes used in this work were 7 mm. (inside diameter) by 250 mm. long with a 1 mm. thickness of wall specially designed for our thermal death point work. They were held in a weak hydrochloric acid solution over night and then rinsed several times with tap water. The tubes were then refilled with distilled water and autoclaved at 15 pounds for 30 minutes after which they were rinsed 3 times with distilled water and drained. The dry tubes were wrapped in packages of 10 each with heavy wrapping paper and sterilized at 160 C. for 3 hours. These sterilized tubes were then ready to receive the juice, and the different tests were made. An

examination of the tables in this paper shows that there is no rapid change in the hydrogen-ion concentration of solutions in soft and hard glass tubes until they have been heated.

METHOD

A series of soft and hard glass tubes each containing 5 c.c. of the solution to be tested was sealed and heated at different temperatures. At definite intervals tubes were removed from the constant temperature bath and the hydrogen-ion concentration determined. Duplicate samples were run in every case and in some instances 3 or 4 samples were used and the average obtained. The food juices heated in this work were those pressed from canned corn, peas, spinach, string beans, beets, sweet potatoes and pumpkin. In the case of sweet potatoes and pumpkin, an equal volume of distilled water was added and the resulting juice pressed as in the other juices. It was impossible to obtain the juice from these undiluted canned products due to their heavy consistency. Different mixtures of disodium acid phosphate and potassium diacid phosphate, freshly distilled water, physiologic salt solution, and weak solutions of hydrochloric acid and sodium hydroxide were also heated in these tubes, and the hydrogen-ion concentration determined at definite intervals. The electrometric method was employed exclusively in these determinations and controls used throughout.

CHANGE IN THE HYDROGEN-ION CONCENTRATION OF UNBUFFERED SOLUTIONS DURING HEATING IN SOFT AND HARD GLASS TUBES

The change in the hydrogen-ion concentration of unbuffered aqueous solutions during heating in soft glass tubes was determined by heating freshly distilled water, physiologic salt solution and solutions of hydrochloric acid and sodium hydroxide at 120 C. for 20 and 30 minutes. The effect of heating was noted on unheated solutions and also on the same solutions in soft glass tubes given a preliminary heating of 100 C. for 5 minutes in order to expel the CO_2 in solution. Physiologic salt solution and solutions of hydrochloric acid and sodium hydroxide were heated at 120 C. for 15 and 30 minutes in hard glass tubes to determine the change in the hydrogen-ion concentration during heating.

Table 1 shows that the hydrogen-ion concentration of these unbuffered aqueous solutions changes enormously when heated in soft

and hard glass tubes. There is great variation in the P_H values of individual determinations in the unheated as well as in the heated solutions. This may be due partly to a variation in the individual tubes, which in the case of soft glass contain some soluble alkali and in the case of hard glass a certain amount of acid. Since there are no buffer salts present in these solutions, even a small amount of acid or alkali would greatly affect the hydrogen-ion concentration. The results, however, show an increase in acidity as the heating is prolonged in hard glass tubes while an increase in alkalinity is observed in soft glass tubes.

TABLE 1

Effect of Heating Unbuffered Solutions in Soft Glass

Treatment	P_H Value of Solutions				
	NaCl 0.85%	Distilled H_2O	HCl N/100,000	NaOH N/1,000	NaOH N/100,000
1. 0	6.85	7.28	6.97	9.92 9.38	8.54
2. Heated in sealed tube 120 C. for 20 min.	9.54	10.07	10.00 10.13	9.40 9.30	9.16
3. Heated in sealed tube 120 C. for 30 min.	9.54 9.60	10.01 10.04	9.86 9.88	9.32 9.62	8.91
4. Heated in open tube 100 C. for 5 min.	9.24	8.70	9.67 9.74		
5. Treated as 4 then sealed heated 120 C. for 30 min.	9.61 9.61	10.04	9,98 10.01		

Effect of Heating Unbuffered Solutions in Hard Glass

Treatment	P_H Value of Solutions				
	NaCl 0.85%	HCl N/1.000	HCl N/100,000	NaOH N/1,000	NaOH N/100 000
0	6.96	3.02 3.04	6.50 7.56	10.03 9.20	8.02 7.53
120 C. - 15 min.	5.84 5.02	3.03 3.02	7.19 6.52	9.15 8.73	7.56 7.39
120 C - 30 min.	5.19 4.48	3.00 2.98	7.00 7.10	8 86 8.28	6.13 5 90

These results are given to demonstrate the marked change in the hydrogen-ion concentration of unbuffered aqueous solutions before heating and after heating at 120 C. for varying times. It definitely shows the inability and impracticability of using unbuffered solutions in the determination of thermal death points when it is desired to maintain a constant hydrogen-ion concentration throughout the procedure.

CHANGE IN THE HYDROGEN-ION CONCENTRATION OF PHOSPHATE MIXTURES HEATED AT 120 IN SOFT AND PYREX GLASS TUBES

Mixtures of the secondary and primary phosphates were used in this test and prepared in 200 c c amounts as follows:

M/5 Na_2 HPO_4	M/5 KH_2 PO_4	Approximate P_H
190 c c	10 c c	8.0
120 c c	80 c c	7.0
20 c c	180 c c	6.0
10 c c	190 c c	5.5
2 c c	198 c c	5.0
0 c c	200 c c	4.5

The actual P_H values of these mixtures are shown in table 2.

Table 2 gives the results obtained showing the change in the hydrogen-ion concentration of the different mixtures after exposure at 120 C. for times varying from 5 to 75 minutes, since that was in excess of the time necessary to destroy the most resistant spores in that medium of a hydrogen-ion concentration requiring the longest exposure for complete sterilization. These figures indicate that when these mixtures are heated in hard glass tubes the hydrogen-ion concentration remains constant during the time required for the complete destruction of highly heat resistant spores, whereas when the same mixture is heated in soft glass tubes, a decided increase in alkalinity is observed. This lowering in the hydrogen-ion concentration is much more marked in an alkaline solution, as shown by a difference in the P_H values of 1.14 for a solution of P_H 7.89. In the neutral solution the change is negligible during the entire heating period, the average P_H readings showing only a difference of 0.06. As the more acid mixtures are heated the change again becomes apparent. The differences in the P_H of the unheated acid solutions and those heated for 75 minutes become greater as the more acid mixtures are used. A change of 0.23 is noted in the original solution of P_H 5.86, 0.45 in the original solution of 4.91 and 0.65 in the original solution of 4.51.

There is a slight change in the hydrogen-ion concentration of the more acid mixtures heated in hard glass tubes, a change in the P_H values of 0.04 and 0.05 being noted in the original solutions of P_H values 491 and 4.46, respectively. This change takes place within the first 10 minutes heating and is hardly sufficient to consider since it lies within the range of possible experimental error in the test. Apparently the buffer salts in the other mixtures control the small

amount of acidity produced by heating and prevent a change in the hydrogen-ion concentration.

Since the increase in acidity produced by heating the mixtures irrespective of the glass tubes is not appreciable, the amount of soluble alkali in the soft glass tubes is unaffected except by the action of the buffer salts and the progressive lowering in the hydrogen-ion concentration results. This same effect was noted at temperatures of 100 C. and above. Although no work was done below boiling, it is safe to assume that a similar condition would exist during an equivalent heating. These results below show that hard glass tubes must be used in heating these phosphate mixtures if a constant hydrogen-ion concentration is to be maintained during a long period.

TABLE 2

Effect of Heating Phosphate Mixtures

Container	PH Value of Solutions Heated at 120 C. for											
	0 Min.	5 Min.	10 Min.	15 Min.	20 Min.	25 Min.	30 Min.	40 Min.	45 Min.	50 Min.	60 Min.	75 Min.
Soft glass	7.89		8.02 7.97		8.00 8.05		8.25		8.24 8.60		8.50 8.98	9.02 9.03
Hard glass	7.88	7.85	7.88		7.86	7.89	7.88	7.87	7.86	7.86	7.87	7.87
Soft glass	6.94		6.93 6.94				6.96 6.98		7.0 7.0		6.97 7.16	6.97 7.02
Hard glass	6.94	6.95	6.95	6.94	6.95	6.95	6.95	6.95		6.95	6.94	6.94
Soft glass	5.86	5.88		5.90	5.91	5.95	5.94	5.92		5.97	5.96	6.09
Hard glass	5.88	5.88	5.86		5.87		5.87		5.87		5.87	5.88
Soft glass	4.91	5.10	5.10	5.05	5.02	5.10	5.04	5.20			5.23	5.36
Hard glass	4.91		4.88		4.87		4.86	4.86			4.86	4.87
Soft glass	4.51	4.69	4.60	4.72		4.78	4.80	4.91		5.08	5.02	5.16
Hard glass	4.46		4.39		4.39		4.39		4.39		4.40	4.41

EFFECT ON THE HYDROGEN-ION CONCENTRATION OF CORN AND PEA JUICES HEATED AT 100 C. IN SOFT AND HARD GLASS TUBES

Table 3 shows the change in the hydrogen-ion concentration of corn and pea juices when heated for varying lengths of time at 100 C. in soft and hard glass tubes. Results of duplicate and triplicate samples show a slight variation in individual determinations, but a comparison of the average values demonstrates the effect produced during a prolonged heating.

Heating in soft glass tubes does not produce a uniform dowering or raising of the hydrogen-ion concentration of either of these juices, bnt a similar change is taking place during the same periods. A slight increase in alkalinity is observed within the first few hours, due to the

presence of the soluble alkali in the glass. The hydrogen-ion concentration remains fairly constant for another period during which interval the acid produced by heating these juices is sufficient to neutralize any additional alkali that dissolves out. Prolonged heating increases the amount of acid to such an extent that there is an excess of hydrogen-ions to hydroxyl-ions, and hence an increase in the hydrogen-ion concentration results. During the heating period (24 hours), however, this is not sufficient to differ greatly from the original unheated juices. If heated for an indefinite period, it is assumed that the hydrogen-ion concentration would be greatly affected.

TABLE 3

EFFECT OF HEATING CANNED CORN AND PEA JUICES

	Container	P_H Value of Solutions Heated at 100 C. for							
		0 Hrs.	2 Hrs.	6 Hrs.	10 Hrs.	12 Hrs.	16 Hrs.	20 Hrs.	24 Hrs.
									5.97
		5.98					6.15	5.99	6.00
	Soft glass	5.97	5.94	6.07	6.06	6.05	6.07	6.05	5.96
		6.00	6.05	6.07	6.06	6.05	6.14	5.98	6.06
	Average	5.98	5.99	6.07	6.06	6.05	6.12	6.01	6.00
Corn juice					5.70				
		5.98		5.83	5.76	5.68	5.55		5.46
	Hard glass	5.98	5.85	5.84	5.74	5.70	5.56	5.50	5.46
		5.97	5.85	5.82	5.68	5.70	5.59	5.52	5.46
	Average	5.98	5.85	5.83	5.73	5.69	5.57	5.51	5.46
		5.58		5.64	5.64	5.69		5.55	5.57
	Soft glass	5.58		5.63	5.63	5.65		5.62	5.63
	Average	5.58		5.64	5.64	5.67		5.58	5.60
Pea juice								5.30	
	Hard glass	5.58		5.50	5.38	5.41		5.28	5.26
		5.58		5.50	5.44	5.41		5.29	5.27
	Average	5.58		5.50	5.41	5.41		5.29	5.27

On the other hand, there is a progressive increase in the acidity of these juices when heated in hard glass tubes which results in a steady increase in the hydrogen-ion concentration. In this respect heating these juices in hard glass tubes gives a different effect than heating a highly buffered solution, such as the phosphate mixtures discussed in a previous section.

Although the hydrogen-ion concentration of these juices does not remain constant during heating in either hard or soft glass tubes, yet it is more constant when the latter are used, and it is believed that results obtained in thermal deathpoint determinations in mediums of definite P_H value are more accurate when soft glass tubes are used than when hard glass tubes are used. The same relationship exists at temperatures above 100 C. for periods sufficient to sterilize solutions containing very resistant spores.

EFFECT ON THE HYDROGEN-ION CONCENTRATION OF VEGETABLE JUICES WHEN HEATED IN SOFT AND HARD GLASS TUBES AT 120 C.

Table 4 shows that the hydrogen-ion concentration is not appreciably altered when the juices pressed from canned corn, peas, spinach, string beans, beets, sweet potatoes and pumpkin are heated in soft glass tubes at 120 C. for different times. Heating these juices for similar periods

TABLE 4

EFFECT OF HEATING CANNED VEGETABLE JUICES

Juice	Container	P_H Value of Solutions Heated at 120 C. for					
		0 Min.	10 Min.	20 Min.	30 Min.	45 Min.	60 Min.
Corn	Soft glass	5.98	6.06	6.05	6.01	6.00	6.00
	Hard glass	5.98	5.83	5.69	5.57	5.52	5.46
Pea	Soft glass	5.58	5.60 5.51		5.59		5.56 5.60
	Hard glass	5.58	5.50	5.53	5.49	5.46	5.43
Spinach	Soft glass	4.60	4.61		4.61		4.61 4.61 4.62
	Hard glass	4.60	4.59	4.58	4.58		4.56
String bean	Soft glass	4.46 4.45	4.49 4.51 4.50	4.52 4.49	4.50	4.49 4.50	4.50
	Hard glass	4.46	4.44	4.44	4.42	4.41	4.40
Sweet potato	Soft glass	4.42	4.42 4.55 4.52	4.50 4.46	4.50 4.48 4.51		4.45 4.43
	Hard glass	4.42	4.40	4.40	4.38	4.36	4.34
Beet	Soft glass	4.27	4.28	4.27	4.27	4.26 4.27	4.26 4.27
	Hard glass	4.27	4.24	4.24	4.24	4.22	4.20
Pumpkin	Soft glass	3.97 3.98 3.97	4.02 4.00	4.00	4.00		4.00 3.99
	Hard glass	3.98	3.97	3.99	4.00	3.98	3.98

in hard glass tubes increases their hydrogen-ion concentration, thus making it impossible to determine the thermal death point in these juices at a constant hydrogen-ion concentration. The juice pressed from canned pumpkin is not altered to the extent that the other juices are when heated in hard glass, the results showing a fairly constant hydrogen-ion concentration throughout. The hydrogen-ion concentration of these juices, however, is higher than that of the raw or canned product since they have been resterilized after they were pressed. A greater change would be observed in the hard glass tubes if the hydrogen-ion concentration of the original juice was more nearly that of the

canned product. These results warrant the use of soft glass tubes in the determination of the thermal death points of suspensions heated in the vegetable juices named.

The results thus far reported in this paper on the effect of heating food juices have been obtained on juices that have been pressed from sterile canned foods and resterilized in the autoclave. The initial hydrogen-ion concentration of these juices was considerably higher than that of the raw unprocessed product. It is interesting to note that the same general change in the hydrogen-ion concentration takes place in the raw food juice much lower in acidity than that of the cooked product.

To determine this effect, a sample of corn was cut from the cob, brine consisting of 6% sugar and 1.25% salt added, and the juice squeezed through a straining cloth. The brine content is about the normal amount used in the canning of corn. This juice was distributed in a series of hard and soft glass tubes and heated at 115 and 120 C. for definite intervals from 10 minutes at 120 C. to 120 minutes at 115 C. The color of the product was also observed. The results obtained on the hydrogen-ion concentration are given in table 5.

TABLE 5

Effect of Heating Raw Corn Juice

	P_H Value of Corn Juice Heated at 115 C. for							Heated at 120 C. for			
	0 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	120 Min.	0 Min.	10 Min.	20 Min.	30 Min.
		7.20	6.64		6.55	6.56	6.50		6.88		
	6.85	7.15	6.68	6.56	6.66	6.53	6.48	6.84	6.94	6.72	6.82
Soft glass	6.84	6.83	6.70	6.58	6.62	6.54	6.54	6.87	6.99	6.78	6.80
	6.87	7.14	6.85	6.54	6.54	6.56	6.54	6.85	7.01	6.65	6.76
Average	6.85	7.08	6.72	6.56	6.59	6.55	6.52	6.85	6.96	6.72	6.79
Hard glass	6.86	6.60		6.30		6.15	6.11	6.86	6.45	6.18	6.11

The same general change takes place as in the other juices. Heating in soft glass tubes at 120 up to 30 minutes affects the hydrogen-ion concentration of this juice appreciably. The average P_H values show an initial lowering in the hydrogen-ion concentration and then an increase to value remaining fairly constant. There is some variation in individual determinations. Using hard glass tubes, on the other hand, the acidity greatly increases as the time is prolonged. The color of the juice darkens as the cooking is continued, due to the caramelization of the sugar.

THE THERMAL DEATH POINT OF SPORES IN CORN JUICE HEATED IN SOFT GLASS AND HARD GLASS TUBES AT 100, 115, AND 120 C.

The thermal death point of spores in corn juice was determined according to the method described by Bigelow and Esty.[1] Table 6 shows the comparative results obtained on the time necessary to destroy a known concentration of spores when heated in corn juice in soft and hard glass tubes at 100, 115 and 120 C. It shows conclusively that the time is materially shorter in every case when hard glass tubes are used, due to the progressive increase in acidity obtained during the heating period.

TABLE 6

THERMAL DEATH POINT OF DIFFERENT CONCENTRATIONS OF SPORES (CULTURE NO. 1503) IN CANNED CORN JUICE AT SPECIFIED TEMPERATURES AND RESULTING CHANGE IN PH VALUE

Container	Spores per C c	Heated at	Time Required to Destroy		Initial P_H	Final P_H
			+	—		
Soft glass......	22,000	100 C.	24 hr.	25 hr.	6.00	6.00
Hard glass.....	22,000	100 C.	20 hr.	21 hr.	6.00	5.62
Soft glass......	2,200	100 C.	22 hr.	23 hr.	6.00	6.00
Hard glass.....	2,200	100 C.	19 hr.	20 hr.	6.00	5.62
Soft glass......	14,000	115 C.	105 min.	110 min.	6.13	6.12
Hard glass.....	14,000	115 C.	80 min.	85 min.	6.13	5.70
Soft glass......	1,400	115 C.	90 min.	95 min.	6.13	6.12
Hard glass.....	1,400	115 C.	75 min.	80 min.	6.13	5.70
Soft glass......	22,000	120 C.	30 min.	32 min.	6.13	6.10
Hard glass.....	22,000	120 C.	27 min.	30 min.	6.13	5.70
Soft glass......	2,200	120 C.	25 min.	27 min.	6.13	6.10
Hard glass.....	2,200	120 C.	22 min.	24 min.	6.13	5.70

The results obtained in soft glass tubes are at a nearly constant hydrogen-ion concentration throughout and represent more accurately the actual time required to destroy at a definite hydrogen-ion concentration.

This table also shows the effect of the initial concentration of spores on the time necessary to destroy them. The results tabulated in the column indicating the time required to destroy spores give the last time at which growth occurred represented by a + sign and the first time at which growth failed to occur showing the destruction of all the spores represented by a — sign. The results shown in the column headed final P_H give the P_H value of the corn juice heated for a period sufficient to destroy the known suspension of spores, the time corresponding to that given in the right hand column headed "Time Required to Destroy Spores."

CONCLUSIONS

Heating unbuffered solutions in soft glass tubes greatly affects the hydrogen-ion concentration.

Heating mixtures of Na_2HPO_4 and KH_2PO_4 in hard glass tubes does not affect the hydrogen-ion concentration during the heating, while prolonged heating in soft glass tubes dissolves out alkali in excess of the amount which can be controlled by the buffer salts. Heating an alkaline solution of this mixture in soft glass causes a greater lowering in the hydrogen-ion concentration than acid solutions. A neutral solution does not change appreciably during heating even in soft glass tubes.

Heating the juices pressed from canned corn, peas, string beans, spinach, beets, sweet potatoes, and pumpkin in soft glass tubes affects the hydrogen-ion concentration less than in hard glass tubes.

A longer time is necessary to destroy the same suspension of spores in corn juice if heated in soft glass tubes than in hard glass tubes.

No general statement can be made regarding the relative merits of hard and soft glass tubes in the determination of the thermal death point. The type of glass to be used for this purpose must be determined for each solution.

In thermal death point determinations the hydrogen-ion concentration of the solution must be known during the entire period of heating.